

Ambient Temperature Preservation of Human FFPE Tumor-Derived Nucleic Acids with Encapsulation Technology

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Cache DNA




UHN

Princess
Margaret
Cancer Centre

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Conflict of Interest Disclosure

 The following authors are employees of Cache DNA and hold stock:

- Lee Organick
- Michael Blas
- James Banal



Lee

 The following authors have **no conflicts of interest** to declare:

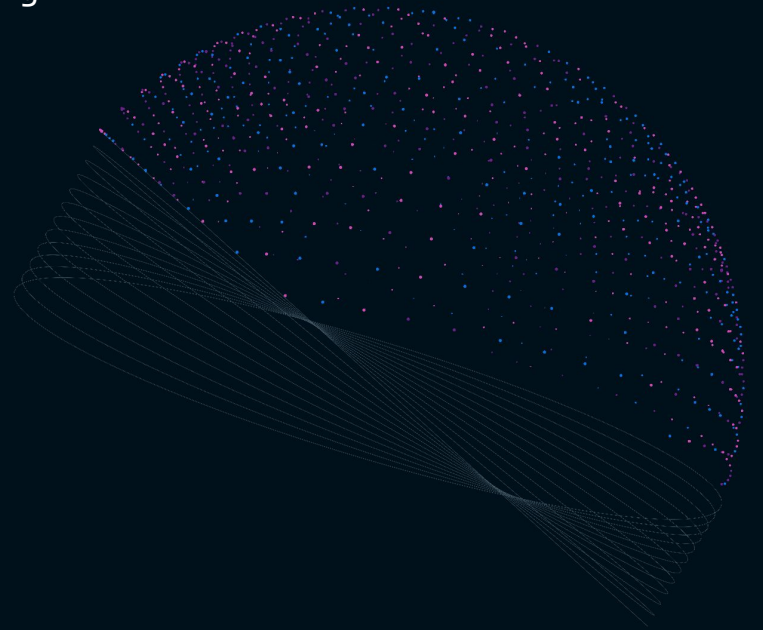
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- Celeste Yu
- Farnoosh Abbas-Aghababazadeh
- Philippe L. Bedard



Farnoosh

Agenda: Ambient Temperature Preservation of Nucleic Acids

- What technical challenges are biobanks currently facing?
- Introduction to Caching
- Collaboration with UHN and Cache
- Questions



What technical challenges are biobanks currently facing?

1. Freezer logistics

- Maintenance
- Monitoring and responses
- Redundant aliquots to prevent freeze-thaws
- Emergencies and accidents



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INVESTIGATES

Email: Unreported freezer failure could have jeopardized all evidence at Austin DNA lab

Eric Dexheimer edexheimer@statesman.com
Published 11:01 p.m. CT Jan. 17, 2017 | Updated 12:27 a.m. CT Sept. 22, 2018

Re: Labs -80 Freezers

ML [Redacted]

Jul 6

Hi all,

We have had several -80s go down due to heat and humidity. Do any of you have room in your -80 that if it was needed we could borrow? Even if it is just one shelf?

Britt Steed, M.Sc. · 2nd
Business Development, Senior Manager | Pharma Supp...
1mo · 📧

This is a self-serving post, but it is also a public service announcement.

Karolinska Institute had a major LN2 storage failure over their Christmas break.

This sounds like a NIGHTMARE. Senior PIs & group leaders losing years' worth of samples, and all because of a cascade of failures:

- ✗ - LN2 automatic replenishment failed
- ✗ - Alarm system only sent emails, and failed to send text alerts
- ✗ - Prior settings had been sensitive enough to cause multiple false alarms, so staff had alarm fatigue
- ✗ - It happened over a holiday

This was all from a system that had passed an inspection from the Manufacturer just a month before.

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Janitor heard 'annoying alarms' and turned off freezer, ruining 20 years of school research worth \$1 million, lawsuit says

By Jessica Xing, CNN
🕒 3 minute read · Updated 11:34 AM EDT, Wed June 28, 2023

isber

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Brain tissue used to study autism destroyed by hospital freezer fault

Leading scientist says loss could set autism research back 10 years following failure of freezer at McLean hospital near Boston

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AL Oct 30, 2023 4:40 PM

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Ancient Arctic ice cores damaged in U of A freezer failure

Nearly 13 per cent of the ice cores have melted, a blow to climate-change research

CBC News · Posted: Apr 06, 2017 11:11 AM PDT | Last Updated: April 6, 2017

I am moving a ~150 freezer (mechanical -80 C) biobank from one location to another approximately 0.5KM away. Looking for guidance in room design and especially temp monitoring systems. Currently, we are using a wired ELPRO ecolog-net system and looking for a system for monitoring with SMS/email alert distribution.

Thankfully,

A [Redacted]

• This article is more than 1 month old

Decades of research destroyed after freezer fails at Swedish university

Estimated value of the samples thought to be in the millions as incident reported to police

What technical challenges are biobanks currently facing?

1. Freezer logistics
 - Maintenance
 - Monitoring and responses
 - Emergencies and accidents
 - Redundant aliquots to prevent freeze-thaws
2. Running out of space or electrical capacity
3. Nucleic acids degrade over time
 - Main causes of degradation are water and air¹
 - DNA in FFPE degrades 2x faster than extracted DNA²

1. Colotte *et al.* Adverse Effect of Air Exposure on the Stability of DNA Stored at Room Temperature. *Biopreservation and Biobanking*, <https://doi.org/10.1089/bio.2010.0028>

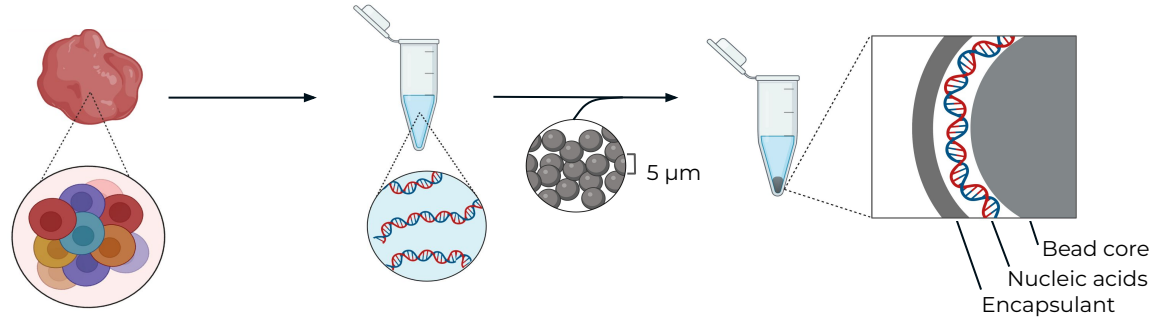
2. Guyard *et al.* DNA degrades during storage in formalin-fixed and paraffin-embedded tissue blocks. *Virchows Arch* 471, 491–500 (2017). <https://doi.org/10.1007/s00428-017-2213-0>

What's wrong with alternative ambient temperature nucleic acid preservation methods?

There are very few storage solutions available. Most are not scalable (to store or retrieve), too expensive, or do not offer adequate protection.

	Additives	Lyophilization	Micro-scale encapsulation	Macro-scale encapsulation
Storage Method	Mix in additive Optional: dehydrate	Complex configurations Specialized equipment Large footprint	Multi-step process Standard lab equipment Benchtop footprint	Samples must be shipped to encapsulator
Recovery Method	Rehydration (1-10 minutes)	Rehydration (1-10 minutes)	De-encapsulation (10-15 minutes)	De-encapsulation (5-10 minutes)
Can Take Aliquots	No	No	Yes	No
Slows Degradation	Only in low-humidity conditions	Yes Likely 10s-100s of years	Yes 10s-100s of years	Yes 1000s of years
Scalable	No	Maybe	Yes	No

Caching nucleic acids



**1. Nucleic acids
are extracted**

**2. Beads and encapsulant
are introduced**

**3. Nucleic acids are
encapsulated**

Peer-reviewed publications on proof-of-concept

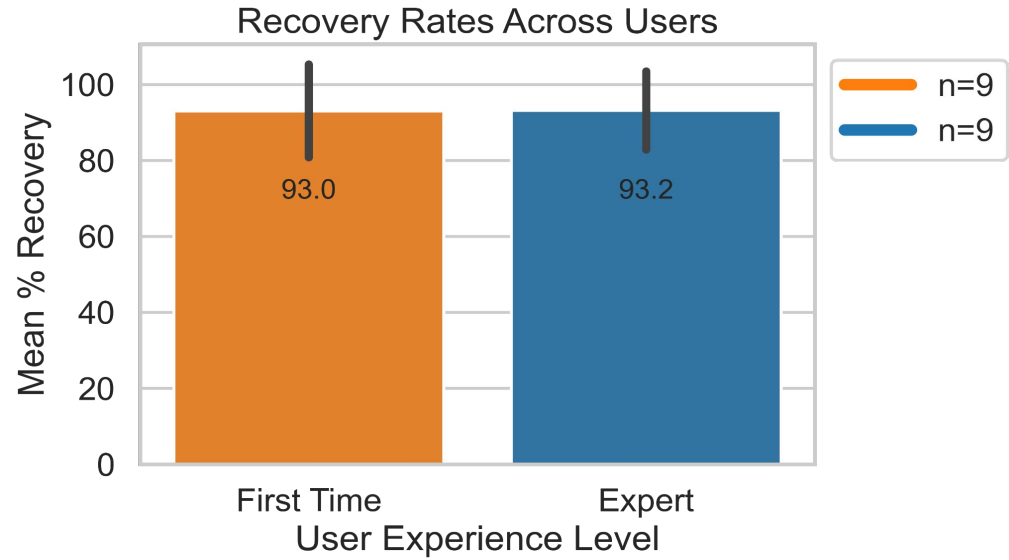
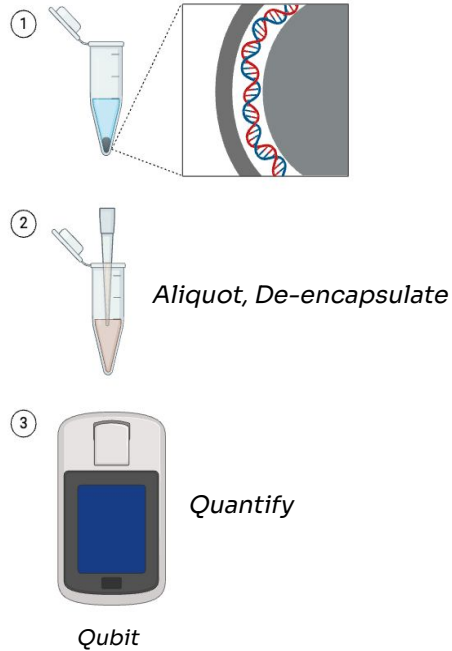
[10.1021/acsami.1c14985](https://doi.org/10.1021/acsami.1c14985)
[10.1038/s41563-021-01021-3](https://doi.org/10.1038/s41563-021-01021-3)

Storage of genomes & RNA
Random access retrieval

Intellectual Property

Growing patent portfolio supporting core platform technology
Cache DNA, Inc. has rights to the methods described herein

Recovery Rate



Note: Recent Caching of FFPE-derived DNA shows mean recovery rate of 93%.

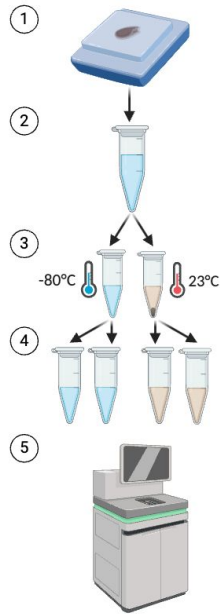
Partnering with University Health Network, Princess Margaret Cancer Research Centre (PM)

Cache is committed to preserving nucleic acids **without compromising quality**. We understand these **samples are precious**, vital for **patient well-being**, and key to **enabling research** to help future patients.

We have partnered with PM to:

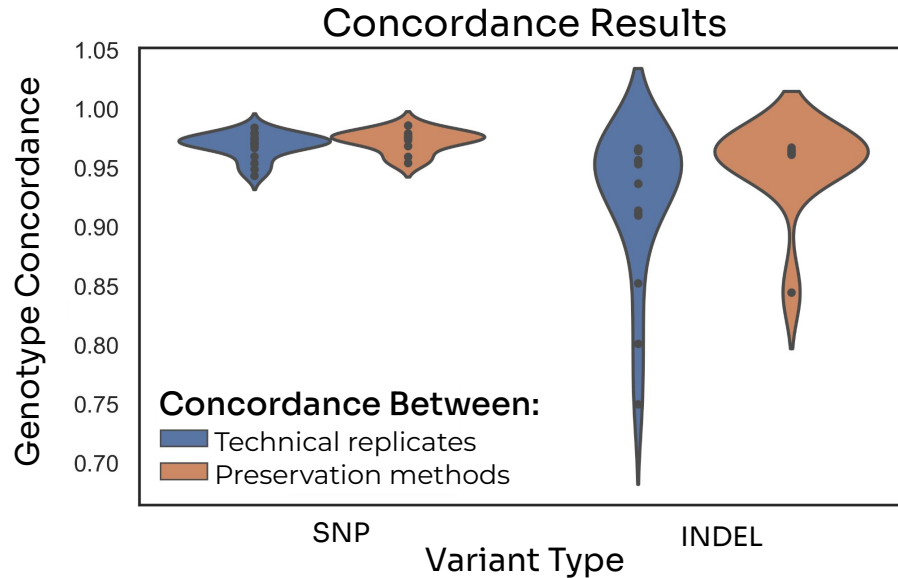
1. **Encapsulate a greater diversity** of nucleic acid origins
2. Perform a **rigorous evaluation of sequence-level changes**

Aim 1 Experimental Overview



1. 10 formalin fixed paraffin embedded (FFPE) tumor samples
2. DNA was extracted and shipped to Cache.
3. Half of each sample was kept frozen, half was Cached.
4. After 1 month, samples were retrieved and split as technical replicates.
5. All 40 samples were prepared for whole genome sequencing (WGS) and sequenced with a NovaSeq.

Result: Sequence Concordance Established



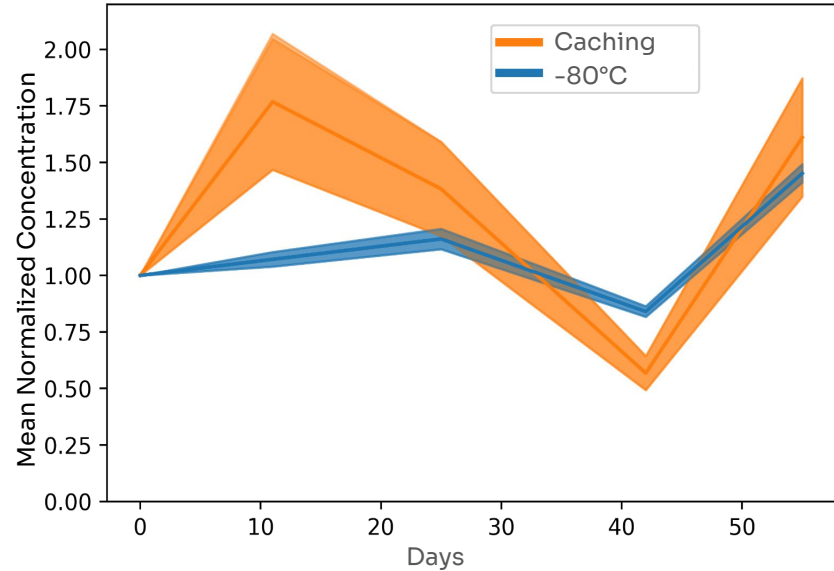
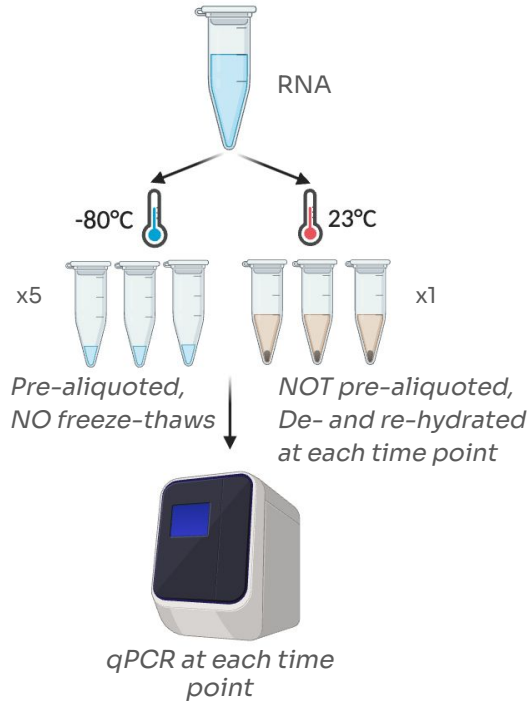
Please Note:

- Caching does not alter DNA sequences.
- We show whole genome sequence concordance between -80°C and Caching.

The Mutect2 variant caller was used on each sample to evaluate:

- Technical replicate concordance
- Preservation method concordance

Preliminary Data Motivating Aim 2 (RNA)

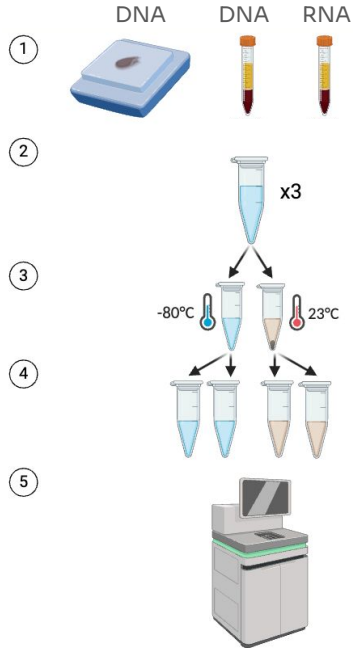


Caching successfully preserves RNA with no measurable degradation after 2 months.

Successful Completion Leads to Aim 2:

1. Caching is a **modular** part of UHN's typical pipeline
 - Utilizing the UHN whole genome and transcriptome sequencing (**WGTS**) pipeline
 - Evaluate **concordance**
2. Caching at **scale**
3. Caching of **RNA** clinical samples

Aim 2 Experimental Overview



1. 19 patients identified. Two DNA samples (paired tumor-normal), one RNA sample per patient.
2. DNA/RNA extracted and shipped to Cache.
3. Half of each sample kept frozen, half Cached.
4. After 1 month, samples retrieved and split in two for technical replicates.
5. All 57 (19 x 3) samples and their replicates (a total of 114 samples) shipped to UHN lab and run through their WGTS pipeline.

Summary

1. Cache preserves DNA and RNA at room temperature for long periods of time and does not compromise on quality
2. Cache is working with UHN to determine sequence concordance for DNA and RNA
 - We have found whole genome sequencing concordance between -80°C and Caching
3. Unless **you** act, current problems with nucleic acid preservation will persist. We want to live in a world with no freezers. Join us!



1. Apply for
your kit
(1 min)

2. Receive
your
invitation

3. Schedule a
consultation
(30 min Zoom)

4. Receive
your early
access kit

5. Share your
experience
(45 min Zoom)

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